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<p>(54) Title: LEPTIN RECEPTOR VARIANTS</p> <p>(57) Abstract</p> <p>The present invention relates to a variant form of the receptor for the <i>obese</i> gene product. In particular, the invention relates to methods of detecting this receptor variant in cells and tissues of obese individuals. In addition, it relates to methods of inhibiting or down-regulating expression of this variant in cells to augment their responsiveness to weight regulation by leptin as well as methods of using compounds to directly activate signal transduction pathways associated with this ligand-receptor system.</p>		

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"LEPTIN RECEPTOR VARIANTS"

1. INTRODUCTION

5 The present invention relates to a variant form of the
receptor for the obese gene product. In particular, the
invention relates to methods of detecting this receptor
variant in cells and tissues of obese individuals. In
10 addition, it relates to methods of inhibiting or down-
regulating expression of this variant in cells to augment
their responsiveness to weight regulation by leptin as well
as methods of using compounds to directly activate signal
transduction pathways associated with this ligand-receptor
15 system.

2. BACKGROUND OF THE INVENTION

Obesity is not only a nutritional disorder in Western
societies, it is also a serious health concern because of its
20 association with adult-onset diabetes, hypertension, and
heart disease (Grundy, 1990, *Disease-a-Month* 36:645-696).
While there was evidence to suggest that body weight was
physiologically regulated, the molecular mechanism has
remained elusive. However, animal studies have produced
25 several mouse strains that contain single-gene mutations,
resulting in an obese phenotype. One such recessive mutation
is manifested in the *ob/ob* mice, and it is referred to as the
obese (ob) mutation.

Zhang et al. (1994, *Nature* 372:425-432) describe the
30 cloning and sequencing of the mouse *ob* gene and its human
homolog. When an isolated gene fragment was used as a probe,
it was shown to hybridize with RNA only in white adipose
tissue by northern blot analysis, but no expression was
detected in any other tissue. In addition, the coding
35 sequence of the *ob* gene hybridized to all vertebrate genomic
DNAs tested, indicating a high level of conservation of this
molecule among vertebrates. The deduced amino acid sequences

are 84% identical between human and mouse, and both molecules contain features of secreted proteins.

In an effort to understand the physiologic function of the *ob* gene, several independent research groups produced 5 recombinant *ob* gene product in bacteria for *in vivo* testing (Pelleymounter et al., 1995, *Science* 269:540-543; Halaas et al., 1995, *Science* 269:543-546; Campfield et al., 1995, *Science* 269:546-549). When the Ob protein (also known as leptin) was injected into grossly obese mice, which possessed 10 two mutant copies of the *ob* gene, the mice exhibited a reduced appetite and began to lose weight. In addition, these studies described a dual action of leptin in both reducing the animals' food intake and in increasing their energy expenditure. Similarly, when normal mice received 15 leptin, they also ate less than the untreated controls. More importantly, Campfield et al. (1995, *Science* 269:546-549) injected leptin directly into lateral ventricle, and observed a reduction in the animals' food intake, suggesting that leptin acts on central neuronal networks to regulate feeding 20 behavior and energy balance. Thus, this result provides evidence that the leptin receptor (also known as OB-R) is expressed by cells in the brain.

Recently, a leptin fusion protein was generated and used to screen for OB-R in a cDNA expression library prepared from 25 mouse choroid plexus, a tissue that lines brain cavities termed ventricles (Tartalia, 1995, *Cell* 83:1263-1271). This approach led to the cloning of one form of the OB-R coding sequence, which reveals a single membrane-spanning receptor, sharing structural similarities with several Class I cytokine 30 receptors, such as the gp130 signal-transducing component of the interleukin-6 receptor (Taga et al., 1989, *Cell* 58:573-581), the granulocyte-colony stimulating factor receptor (Fukunaga et al., 1990, *Cell* 61:341-350), and the leukemia inhibitory factor receptor (Gearing et al., 1991, *EMBO J.* 35 10:2839-2848). Northern blot analysis and reverse transcription-polymerase chain reaction (RT-PCR) demonstrate

that OB-R mRNA is expressed in several tissues, including lung, kidney, total brain, choroid plexus and hypothalamus.

The reported mouse OB-R protein contains a relatively short intracellular cytoplasmic domain as compared with other Class I cytokine receptors. Subsequently, when cDNA encoding its human homolog was isolated from a human infant brain library, the predicted human protein sequence contains a much longer intracellular domain. In view of this finding, it was speculated that different forms of the receptor might exist (Barinaga, 1996, *Science* 271:29). However, prior to the present invention, there was no report on the identification of any variant forms of the OB-R in humans or how such molecules, if they exist, would relate to obesity.

Additionally, several studies have shown that *ob* gene expression is actually increased in obese humans (Considine et al., 1995, *J. Clin. Invest.* 95:2986-2988; Lonnquist et al., 1995, *Nature Med.* 1:950; Hamilton et al., 1995, *Nature Med.* 1:953). Moreover, the mutations in the mouse *Ob* gene were not detected in human mRNA. Therefore, taken collectively, these studies imply that decreased leptin levels are not the primary cause of obesity, and argue for the presence of a less responsive receptor in obese individuals. There remains a need to isolate such an OB-R variant for the design of therapeutics to augment weight regulation by leptin.

3. SUMMARY OF THE INVENTION

The present invention relates to a variant form of the human OB-R. In particular, it relates to the detection of this receptor variant in cells of obese individuals, and methods for treating obesity by targeting this variant.

The invention is based, in part, upon the Applicants' discovery of human cDNA clones encoding a variant form of the OB-R. This receptor differs structurally from a reported OB-R with only three amino acid substitutions in the extracellular domain, but extensive diversity is observed in their intracellular cytoplasmic domains at the 3' end. The

cytoplasmic domain of the variant of the invention is both shorter and distinct in nucleotide sequence from the corresponding domain of the published form of OB-R.

Therefore, a wide variety of uses are encompassed by the present invention, including but not limited to, the detection of the receptor variant in cells of obese individuals, methods to inhibit and/or down-regulate the expression of this receptor variant, gene therapy to replace the receptor variant in homozygous individuals, and direct activation of downstream signal transduction pathways in cells expressing the receptor variant for weight regulation.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-1E. Nucleotide sequence and deduced amino acid sequence of the human OB-R variant. The amino acid sequence diverges from the human OB-R reported by Tartaglia et al. (1995, Cell 83:1263-1271) at nucleotide residue #349, #422, #764 and from residue #2770 and beyond.

20

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. THE OB-R VARIANT

The present invention relates to nucleic acid and amino acid sequences of an OB-R variant in the Class I cytokine receptor family. In a specific embodiment by way of example in Section 6, *infra*, this variant was cloned and characterized. Amino acid sequence comparison of this OB-R variant with a published human OB-R sequence (Tartaglia et al., 1995, Cell 83:1263-1271) reveals three amino acid differences in their extracellular domain and extensive diversity in their intracellular cytoplasmic domains. More specifically, Figure 1A-1E shows that in the variant, nucleotide residues #349-351 encode alanine, nucleotide residues #421-423 encode arginine, and nucleotide residues #763-765 encode arginine. Additionally, the variant diverges both in length and sequence composition from the published human OB-R sequence from nucleotide residue #2770 and beyond.

In order to clone additional variant forms of the molecule, labeled DNA probes made from nucleic acid fragments corresponding to any portion of the cDNA disclosed herein may be used to screen a cDNA library prepared from human fetal liver, human lung, human kidney, human choroid plexus and human hypothalamus. More specifically, oligonucleotides corresponding to either the 5' or 3' terminus of the cDNA sequence may be used to obtain longer nucleotide sequences. Briefly, the library may be plated out to yield a maximum of 30,000 pfu for each 150 mm plate. Approximately 40 plates may be screened. The plates are incubated at 37°C until the plaques reach a diameter of 0.25 mm or are just beginning to make contact with one another (3-8 hours). Nylon filters are placed onto the soft top agarose and after 60 seconds, the filters are peeled off and floated on a DNA denaturing solution consisting of 0.4N sodium hydroxide. The filters are then immersed in neutralizing solution consisting of 1M Tris HCL, pH 7.5, before being allowed to air dry. The filters are prehybridized in casein hybridization buffer containing 10% dextran sulfate, 0.5M NaCl, 50mM Tris HCL, pH 7.5, 0.1% sodium pyrophosphate, 1% casein, 1% SDS, and denatured salmon sperm DNA at 0.5 mg/ml for 6 hours at 60°C. The radiolabelled probe is then denatured by heating to 95°C for 2 minutes and then added to the prehybridization solution containing the filters. The filters are hybridized at 60°C for 16 hours. The filters are then washed in 1X wash mix (10X wash mix contains 3M NaCl, 0.6M Tris base, and 0.02M EDTA) twice for 5 minutes each at room temperature, then in 1X wash mix containing 1% SDS at 60°C for 30 minutes, and finally in 0.3X wash mix containing 0.1% SDS at 60°C for 30 minutes. The filters are then air dried and exposed to x-ray film for autoradiography. After developing, the film is aligned with the filters to select a positive plaque. If a single, isolated positive plaque cannot be obtained, the agar plug containing the plaques will be removed and placed in lambda dilution buffer containing 0.1M NaCl, 0.01M magnesium sulfate, 0.035M Tris HCL, pH 7.5, 0.01% gelatin. The phage

may then be replated and rescreened to obtain single, well isolated positive plaques. Positive plaques may be isolated and the cDNA clones sequenced using primers based on the known cDNA sequence. This step may be repeated until a full 5 length cDNA is obtained.

One method for identifying all 3' isoforms is to PCR amplify the 3' ends of the variant cDNA from a variety of tissues including but not limiting to, choroid plexus, hypothalamus, fetal liver, bone marrow, ovary, or prostate. 10 To obtain the 3' end of the cDNA, an oligo-dT primer is used to synthesize the cDNA first strand. OB-R specific primers from the conserved region of the gene (e.g. up stream of nucleotide 2770) and oligo-dT are then used to amplify the 3' end. The PCR fragments are cloned and sequenced by standard 15 techniques. Once obtained, these sequences may be translated into amino acid sequence and examined for certain landmarks such as continuous open reading frame, regulatory regions that associate with tyrosine kinase activation, and finally overall structural similarity to known OB-R variants.

20

5.2. EXPRESSION OF THE OB-R VARIANT

In accordance with the invention, the OB-R variant polynucleotide sequence which encodes a protein, peptide fragments, fusion proteins or functional equivalents thereof, 25 may be used to generate recombinant DNA molecules that direct the expression of the protein, peptide fragments, fusion proteins or a functional equivalent thereof, in appropriate host cells. Such polynucleotide sequences, as well as other polynucleotides which selectively hybridize to at least a 30 part of such polynucleotides or their complements, may also be used in nucleic acid hybridization assays, Southern and Northern blot analyses, etc.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a 35 functionally equivalent amino acid sequence, may be used in the practice of the invention for the expression of the OB-R variant. Such DNA sequences include those which are capable

of hybridizing to the OB-R variant sequence under stringent conditions, particularly at its 3' end. The phrase "stringent conditions" as used herein refers to those hybridizing conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Altered DNA sequences which may be used in accordance with the invention include deletions, additions or substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product itself may contain deletions, additions or substitutions of amino acid residues within the OB-R variant sequence, which result in a silent change thus producing a functionally equivalent protein. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine, histidine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: glycine, asparagine, glutamine, serine, threonine, tyrosine; and amino acids with nonpolar head groups include alanine, valine, isoleucine, leucine, phenylalanine, proline, methionine, tryptophan.

The DNA sequence of the invention may be engineered in order to alter the OB-R variant coding sequence for a variety of ends, including but not limited to, alterations which

modify processing and expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc. In addition, the intracellular domain may also be altered and replaced by a different domain, such as the OB-R intracellular domain by Tartaglia et al.

In another embodiment of the invention, the OB-R variant sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors or stimulators of receptor activity, it may be useful to encode a chimeric protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the OB-R variant sequence and the heterologous protein sequence, so that the variant may be cleaved away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of the OB-R variant could be synthesized in whole or in part, using chemical methods well known in the art. (See, for example, Caruthers et al., 1980, *Nuc. Acids Res. Symp. Ser.* 7:215-233; Crea and Horn, 180, *Nuc. Acids Res.* 9(10):2331; Matteucci and Caruthers, 1980, *Tetrahedron Letters* 21:719; and Chow and Kempe, 1981, *Nuc. Acids Res.* 9(12):2807-2817). Alternatively, the protein itself could be produced using chemical methods to synthesize OB-R variant amino acid sequence in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (e.g., see Creighton, 1983, *Proteins Structures And Molecular Principles*, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 34-49).

In order to express the OB-R variant in host cells, the nucleotide sequence coding for the variant, or a functional equivalent, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. The expressed gene products as well as host cells or cell lines transfected or transformed with recombinant OB-R variant expression vectors can be used for a variety of purposes. For example, host cells expressing the OB-R variant may be used to verify the ability of this molecule to bind leptin in a binding assay with radiolabeled, enzyme-conjugated or fluorescent dye-conjugated leptin. At the same time, the ability of the molecule to transduce an activation signal in host cells upon binding to leptin may be tested by assaying proliferation or phosphorylation pattern of kinases in the cells. In addition, genetically-engineered host cells can be used to screen for and select agonist and antagonist compounds, including any inhibitors that would interfere with binding of leptin to the extracellular domain of the OB-R variant. In that connection, such host cells may be used to screen for and select small molecules that can supplement the incomplete signal transduced by the OB-R variant following leptin binding. Other uses, include, but are not limited to generating antibodies (i.e., monoclonal or polyclonal) that competitively inhibit activity of an OB-R variant, neutralize its activity, or even enhances its activity. Antibodies may be used in detecting and quantifying expression of OB-R levels in cells and tissues.

5.3. USES OF THE OB-R VARIANT POLYNUCLEOTIDE

The OB-R variant polynucleotide may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, the OB-R variant polynucleotide may be used to detect gene expression or aberrant gene expression in obese individuals as well as in normal individuals to identify predisposition for obesity. Included in the scope of the invention are oligonucleotide sequences, that include

antisense RNA and DNA molecules, ribozymes and triplex DNA, that function to inhibit translation of OB-R variant.

5.3.1. DIAGNOSTIC USES OF OB-R VARIANT POLYNUCLEOTIDE

5 The OB-R variant polynucleotide may have a number of uses for the diagnosis of the possible causes underlying obesity, resulting from expression of the receptor variant. For example, the OB-R variant cytoplasmic domain DNA sequence may be used in hybridization assays of biopsies or autopsies
10 to diagnose OB-R variant expression; e.g., Southern or Northern analysis, including *in situ* hybridization assays as well as PCR. Such techniques are well known in the art, and are in fact the basis of many commercially available diagnostic kits. For PCR detection, primers may be designed
15 from a conserved region of the coding sequence and within the 3' region of OB-R variant. The tissues suitable for such analysis include but are not limited to, hypothalamus, choroid plexus, adipose tissues, lung, prostate, ovary, small intestine, bone marrow and peripheral blood mononuclear
20 cells.

5.3.2. THERAPEUTIC USES OF THE OB-R VARIANT POLYNUCLEOTIDE

The OB-R variant polynucleotide may be useful in the
25 treatment of various abnormal obese conditions. By introducing gene sequences into cells, gene therapy can be used to treat conditions in which the cells do not respond to leptin normally due to expression of the OB-R variant. In some instances, the polynucleotide encoding a functional OB-R
30 is intended to replace or act in the place of the functionally deficient OB-R variant gene. Alternatively, abnormal conditions characterized by expression of two copies of the OB-R variant can be treated using the gene therapy techniques described below.

35 Non-responsiveness to normal levels of leptin is an important cause of obesity. This may result from a functionally defective receptor that does not transduce

competent signals upon ligand binding. Recombinant gene therapy vectors, such as viral vectors, may be engineered to express signalling competent forms of OB-R which may be used to augment the non-responsiveness of the naturally occurring
5 OB-R variant. A signalling competent form may be, for example, a protein with the same extracellular domain and transmembrane region, but containing all or part of its normal signal transduction domain, such as that described by Tartaglia et al. (1995, Cell 83:1263-1271). Thus recombinant
10 gene therapy vectors may be used therapeutically for treatment of obesity resulting from expression or activity of the OB-R variant. Accordingly, the invention provides a method of augmenting signal transduction by an endogenous OB-R variant in a cell comprising delivering a DNA molecule
15 encoding a signalling competent form of the OB-R to the cell so that the signalling competent protein is produced in the cell and competes with the endogenous defective OB-R variant for access to molecules in the signalling pathway which does not activate or are not activated by the endogenous natural
20 defective receptor. Additionally, since dimerization of a functional receptor with a defective variant may occur in cells of heterozygous individuals, small molecules may be used to inhibit such pairing, thereby increasing the number of functional dimeric receptors for proper signalling in
25 response to leptin.

In contrast, overexpression of either leptin or a competent OB-R may result in a clinical anorexic-like syndrome due to a loss of appetite or hypermetabolic activity. In such cases, the OB-R variant of the invention
30 may be introduced into cells with functional receptors to cause a decrease in the number of functional receptors or to compete with such receptors for leptin binding.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes
35 viruses, or bovine papilloma virus, may be used for delivery of recombinant functional OB-R into the targeted cell population. Methods which are well known to those skilled in

the art can be used to construct recombinant viral vectors containing an OB-R polynucleotide sequence. See, for example, the techniques described in Sambrook et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. Alternatively, recombinant OB-R molecules can be reconstituted into liposomes for delivery to target cells.

10 Oligonucleotide sequences including anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of the OB-R variant mRNA are within the scope of the invention. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted
15 mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the OB-R variant nucleotide sequence at nucleotide #2771 and beyond, are preferred.

Ribozymes are enzymatic RNA molecules capable of
20 catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules
25 that specifically and efficiently catalyze endonucleolytic cleavage of OB-R variant RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the
30 following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the
35 oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their

accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Oligodeoxyribonucleotides can form sequence-specific triple helices by hydrogen bonding to specific complementary sequences in duplexed DNA. Interest in triple helices has focused on the potential biological and therapeutic applications of these structures. Formation of specific triple helices may selectively inhibit the replication and/or gene expression of targeted genes by prohibiting the specific binding of functional trans-acting factors.

Oligonucleotides to be used in triplex helix formation should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Oligonucleotide sequences may be pyrimidine-based, which will result in TAT and CGC triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich oligonucleotides provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, oligonucleotides may be chosen that are purine-rich, for example, containing a stretch of G residues. These oligonucleotides will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex. Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" oligonucleotide.

Switchback oligonucleotides are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

5.4. ACTIVATION OF TYROSINE KINASE PATHWAYS IN OBESITY

Many known class I cytokine receptors initiate cell signaling via Janus kinases (JAKs) (Ihle, 1995, *Nature* 377:591-594; Heldin, 1995, *Cell* 80:213-223; Kishimoto et al, 1994, *Cell* 76:253-62; Ziemiecki et al, 1994, *Trends Cell Biol.* 4:207-212). JAK1-3 have been shown to bind to conserved sequences termed box1 and box2 (Fukunaga et al., 1991, *EMBO J.* 10:2855-65; Murakami, 1991, *Proc. Natl. Acad. Sci. USA* 88:11349-53). Ligand binding induces a homo- or hetero-dimerization of receptor chains which activates, by phosphorylation, the JAKs. The activated JAKs, in turn, phosphorylate members of the STAT family (Heldin, 1995, *Cell* 80:213-223; Kishimoto et al., *Blood* 86:1243-54; Darnell et al., 1994, *Science* 264:1415-21; Zhong et al, 1994, *Proc.*

Natl. Acad. Sci. USA 91:4806-10; Hou et al., 1994, Science 265:1701-6). These phosphorylated STATs ultimately translocate to the nucleus, form transcription complexes, and regulate gene expression. Both box1 and box2 are required 5 for complete signaling in certain systems. (Fukunaga et al., 1991, EMBO J. 10:2855-65; Murakami, 1991, Proc. Natl. Acad. Sci. USA 88:11349-53). The OB-R variant disclosed herein has a typical box1 (from nucleotide #2707-2730) that contains the critical xWxxxPxP amino acid sequence, but it does not 10 contain an obvious box2 nor further downstream sequences that are important for normal receptor activation. Therefore, it is possible to use compounds that activate JAKs to directly activate these pathways for weight regulation without triggering the OB-R.

15

6. EXAMPLE: MOLECULAR CLONING OF AN OB-R VARIANT

A number of cDNA clones were isolated from a human fetal liver cDNA library (Clontech, Palo Alto, CA), and the DNA sequences of several of these clones were determined. These 20 clones (designated as Hu-B1.219 #4, #33, #34, #1, #3, #57, #62) contained overlapping sequences, which were then compiled into a contiguous nucleotide sequence (Figure 1A-1E). When the deduced amino acid sequence of one such sequence was compared with the sequence of a recently 25 published human OB-R, they were shown to be nearly identical in the extracellular domains with the exception of three amino acids, whereas there existed extensive diversity in their intracellular cytoplasmic domains at the 3' end. The predicted protein sequence contains two FN III domains, each 30 containing a "WS box", which are characteristic of genes of the Class I cytokine receptor family. Therefore, the cDNA disclosed herein encodes an OB-R variant.

When various human tissue RNA were probed with a fragment of this OB-R variant by Northern blot analysis, 35 expression of this molecule was detected in heart, placenta, lung, liver, muscle, pancreas, prostate, ovary, small intestine and brain.

Based on the sequence presented in Figure 1A-1E, the translation initiation site appears at position #97. The sequence encodes an open reading frame up to and including nucleotide #2784. It is believed that the sequence between 5 nucleotides #2629 and #2682 encodes a transmembrane domain. The complete sequence encodes a protein of 896 amino acids.

The sequence of the OB-R variant is identical to the sequence of human OB-R reported by Tartaglia (1995, Cell 83:1263-1271) in the transmembrane region and a portion of 10 the intracellular domain up to and including nucleotide #2769, then they diverge at nucleotide #2770 and beyond. In addition, the product of this cDNA is substantially shorter in its intracellular domain than the published human OB-R. These two forms of OB-R may derive from a common precursor 15 mRNA by an alternative splicing mechanism. The sequence in this region is consistent with well known splice junctions.

7. DEPOSIT OF MICROORGANISMS

The following organisms were deposited with the American 20 Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

<u>Strain Designation</u>	<u>Accession No.</u>
HuB1.219, #1	75885
HuB1.219, #4	75886
25 HuB1.219, #33	75888
HuB1.219, #34	75889
HuB1.219, #3	75970
HuB1.219, #57	75972
HuB1.219, #62	75974

The present invention is not to be limited in scope by 30 the exemplified embodiments, which are intended as illustrations of individual aspects of the invention. Indeed, various modifications for the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and 35 accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications cited herein are incorporated by reference in their entirety.

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International Application No: PCT/ /

MICROORGANISMSOptional Sheet in connection with the microorganism referred to on page 16, lines 17-37 of the description ***A. IDENTIFICATION OF DEPOSIT ***

Further deposits are identified on an additional sheet *

Name of depositary institution *

American Type Culture Collection

Address of depositary institution (including postal code and country) *

12301 Parklawn Drive
Rockville, MD 20852
USDate of deposit * September 14, 1994 Accession Number * 75885**B. ADDITIONAL INDICATIONS** * (leave blank if not applicable). This information is contained on a separate attached sheet**C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *** if the indications are not all designated States**D. SEPARATE FURNISHING OF INDICATIONS *** (leave blank if not applicable)The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g.,
"Accession Number of Deposit")E. ☐ This sheet was received with the International application when filed (to be checked by the receiving Office)_____
(Authorized Officer)☐ The date of receipt (from the applicant) by the International Bureau *

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
US

<u>Accession No.</u>	<u>Date of Deposit</u>
75886	September 14, 1994
75888	September 14, 1994
75889	September 14, 1994
75970	December 14, 1994
75972	December 14, 1994
75974	December 14, 1994

WHAT IS CLAIMED IS:

1. A method for detecting a defective OB-R in cells comprising:
 - (a) extracting RNA from a cell population;
 - 5 (b) contacting the RNA with an oligonucleotide derived from a portion of the sequence depicted in Figure 1A-1E; and
 - (c) detecting hybridization of the RNA with the oligonucleotide.
- 10 2. The method of Claim 1 in which the cell population is obtained from the brain.
3. The method of Claim 1 in which the cell population
15 is obtained from the lung.
4. The method of Claim 1 in which the cell population is obtained from the kidney.
- 20 5. The method of Claim 1 in which the oligonucleotide is derived from nucleotide residue #2770 and beyond in the sequence depicted in Figure 1A-1E.
6. A method for treating obesity, comprising
25 administering to an individual an effective amount of an agent capable of inhibiting expression of an OB-R variant gene.
7. The method of Claim 6 in which the OB-R variant
30 gene further comprises the sequence of Figure 1A-1E or which is capable of selectively hybridizing to it.
8. The method of Claim 7 in which the agent is an antisense molecule complementary to mRNA encoded by the
35 sequence of Figure 1A-1E.

9. The method of Claim 7 in which the agent is a ribozyme molecule specific for mRNA encoded by the sequence of Figure 1A-1E.

5 10. The method of Claim 7 in which the agent is a triple helix component.

11. A method for identifying a compound capable of supplementing biological activity of leptin, comprising:

- 10 (a) incubating host cell expressing an OB-R variant with leptin;
- (b) incubating a portion of the leptin-treated cells with a test compound; and
- (c) comparing activation signal in the cells
- 15 treated in step (b) with cells treated in step (a);

thereby determining whether the compound augments activation of the OB-R variant by leptin.

20 12. The method of Claim 11 in which the OB-R variant is encoded by the sequence depicted in Figure 1A-1E.

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9	18	27	36	45	54
GCG CGC GCG ACG CAG GTG CCC GAG CCC CGG CCC GCG CCC ATC TCT GCC TTC GGT					
A R A T Q V P E P R P A P I S A F G					
63	72	81	90	99	108
CGA GTT GGA CCC CCG GAT CAA GGT GTA CTT CTC TGA AGT AAG ATG ATT TGT CAA					
R V G P P D Q G V L L * S K M I C Q					
117	126	135	144	153	162
AAA TTC TGT GTG GTT TTG TTA CAT TGG GAA TTT ATT TAT GTG ATA ACT GCG TTT					
K F C V V L L H W E F I Y V I T A F					
171	180	189	198	207	216
AAC TTG TCA TAT CCA ATT ACT CCT TGG AGA TTT AAG TTG TCT TGC ATG CCA CCA					
N L S Y P I T P W R F K L S C M P P					
225	234	243	252	261	270
AAT TCA ACC TAT GAC TAC TTC CTT TTG CCT GCT GGA CTC TCA AAG AAT ACT TCA					
N S T Y D Y F L L P A G L S K N T S					
279	288	297	306	315	324
AAT TCG AAT GGA CAT TAT GAG ACA GCT GTT GAA CCT AAG TTT AAT TCA AGT GGT					
N S N G H Y E T A V E P K F N S S G					
333	342	351	360	369	378
ACT CAC TTT TCT AAC TTA TCC AAA GCA ACT TTC CAC TGT TGC TTT CGG AGT GAG					
T H F S N L S K A T F H C C F R S E					
387	396	405	414	423	432
CAA GAT AGA AAC TGC TCC TTA TGT GCA GAC AAC ATT GAA GGA AGG ACA TTT GTT					
Q D R N C S L C A D N I E G R T F V					
441	450	459	468	477	486
TCA ACA GTA AAT TCT TTA GTT TTT CAA CAA ATA GAT GCA AAC TGG AAC ATA CAG					
S T V N S L V F Q Q I D A N W N I Q					
495	504	513	522	531	540
TGC TGG CTA AAA GGA GAC TTA AAA TTA TTC ATC TGT TAT GTG GAG TCA TTA TTT					
C W L K G D L K L F I C Y V E S L F					
549	558	567	576	585	594
AAG AAT CTA TTC AGG AAT TAT AAC TAT AAG GTC CAT CTT TTA TAT GTT CTG CCT					
R N L F R N Y N Y K V H L L Y V L P					
603	612	621	630	639	648
GAA GTG TTA GAA GAT TCA CCT CTG GTT CCC CAA AAA GGC AGT TTT CAG ATG GTT					
E V L E D S P L V P Q K G S F Q M V					

Figure 1A

CAC	TGC	657 AAT	TGC	666 AGT	GTT	CAT	GAA	675 TGT	TGT	GAA	684 TGT	CTT	GTG	693 CCT	GTG	CCA	702 ACA
H	C	N	C	S	V	H	E	C	C	E	C	L	V	P	V	P	T
GCC	AAA	711 CTC	AAC	720 GAC	ACT	CTC	CTT	729 ATG	TGT	TTG	738 AAA	ATC	ACA	747 TCT	GGT	GGA	756 GTA
A	K	L	N	D	T	L	L	M	C	L	K	I	T	S	G	G	V
ATT	TTC	765 CGG	TCA	774 CCT	CTA	ATG	TCA	783 GTT	CAG	CCC	792 ATA	AAT	ATG	801 GTG	AAG	CCT	810 GAT
I	F	R	S	P	L	M	S	V	Q	P	I	N	M	V	K	P	D
CCA	CCA	819 TTA	GGT	828 TTG	CAT	ATG	GAA	837 ATC	ACA	GAT	846 GAT	GGT	AAT	855 TTA	AAG	ATT	864 TCT
P	P	L	G	L	H	M	E	I	T	D	D	G	N	L	K	I	S
TGG	TCC	873 AGC	CCA	882 CCA	TTG	GTA	CCA	891 TTT	CCA	CTT	900 CAA	TAT	CAA	909 GTG	AAA	TAT	918 TCA
W	S	S	P	P	L	V	P	F	P	L	Q	Y	Q	V	K	Y	S
GAG	AAT	927 TCT	ACA	936 ACA	GTT	ATC	AGA	945 GAA	GCT	GAC	954 AAG	ATT	GTC	963 TCA	GCT	ACA	972 TCC
E	N	S	T	T	V	I	R	E	A	D	K	I	V	S	A	T	S
CTG	CTA	981 GTA	GAC	990 AGT	ATA	CTT	CCT	999 GGG	TCT	TCG	1008 TAT	GAG	GTT	1017 CAG	GTG	AGG	1026 GGC
L	L	V	D	S	I	L	P	G	S	S	Y	E	V	Q	V	R	G
AAG	AGA	1035 CTG	GAT	1044 GGC	CCA	GGA	ATC	1053 TGG	AGT	GAC	1062 TGG	AGT	ACT	1071 CCT	CGT	GTC	1080 TTT
K	R	L	D	G	P	G	I	W	S	D	W	S	T	P	R	V	F
ACC	ACA	1089 CAA	GAT	1098 GTC	ATA	TAC	TTT	1107 CCA	CCT	AAA	1116 ATT	CTG	ACA	1125 AGT	GTT	GGG	1134 TCT
T	T	Q	D	V	I	Y	F	P	P	K	I	L	T	S	V	G	S
AAT	GTT	1143 TCT	TTT	1152 CAC	TGC	ATC	TAT	1161 AAG	AAG	GAA	1170 AAC	AAG	ATT	1179 GTT	CCC	TCA	1188 AAA
N	V	S	F	H	C	I	Y	K	K	E	N	K	I	V	P	S	K
GAG	ATT	1197 GTT	TGG	1206 TGG	ATG	AAT	TTA	1215 GCT	GAG	AAA	1224 ATT	CCT	CAA	1233 AGC	CAG	TAT	1242 GAT
E	I	V	W	W	M	N	L	A	E	K	I	P	Q	S	Q	Y	D
GTT	GTG	1251 AGT	GAT	1260 CAT	GTT	AGC	AAA	1269 GTT	ACT	TTT	1278 TTC	AAT	CTG	1287 AAT	GAA	ACC	1296 AAA
V	V	S	D	H	V	S	K	V	T	F	F	N	L	N	E	T	K
OCT	CGA	1305 GGA	AAG	1314 TTT	ACC	TAT	GAT	1323 GCA	GTG	TAC	1332 TGC	TGC	AAT	1341 GAA	CAT	GAA	1350 TGC
P	R	G	K	F	T	Y	D	A	V	Y	C	C	N	E	H	E	C

Figure 1B

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1359	1368	1377	1386	1395	1404
CAT CAT CGC TAT GCT GAA TTA TAT GTG ATT GAT GTC AAT ATC AAT ATC TCA TGT					
H H R Y A E L Y V I D V N I N I S C					
1413	1422	1431	1440	1449	1458
GAA ACT GAT GGG TAC TTA ACT AAA ATG ACT TGC AGA TGG TCA ACC AGT ACA ATC					
E T D G Y L T K M T C R W S T S T I					
1467	1476	1485	1494	1503	1512
CAG TCA CTT GCG GAA AGC ACT TTG CAA TTG AGG TAT CAT AGG AGC AGC CTT TAC					
Q S L A E S T L Q L R Y H R S S L Y					
1521	1530	1539	1548	1557	1566
TGT TCT GAT ATT CCA TCT ATT CAT CCC ATA TCT GAG CCC AAA GAT TGC TAT TTG					
C S D I P S I H P I S E P K D C Y L					
1575	1584	1593	1602	1611	1620
CAG AGT GAT GGT TTT TAT GAA TGC ATT TTC CAG CCA ATC TTC CTA TTA TCT GGC					
Q S D G F Y E C I F Q P I F L L S G					
1629	1638	1647	1656	1665	1674
TAC ACA ATG TGG ATT AGG ATC AAT CAC TCT CTA GGT TCA CTT GAC TCT CCA CCA					
Y T M W I R I N H S L G S L D S P P					
1683	1692	1701	1710	1719	1728
ACA TGT GTC CTT CCT GAT TCT GTG GTG AAG CCA CTG CCT CCA TCC AGT GTG AAA					
T C V L P D S V V K P L P P S S V K					
1737	1746	1755	1764	1773	1782
GCA GAA ATT ACT ATA AAC ATT GGA TTA TTG AAA ATA TCT TGG GAA AAG CCA GTC					
A E I T I N I G L L K I S W E K P V					
1791	1800	1809	1818	1827	1836
TTT CCA GAG AAT AAC CTT CAA TTC CAG ATT CGC TAT GGT TTA AGT GGA AAA GAA					
F P E N N L Q F Q I R Y G L S G K E					
1845	1854	1863	1872	1881	1890
GTA CAA TGG AAG ATG TAT GAG GTT TAT GAT GCA AAA TCA AAA TCT GTC AGT CTC					
V Q W K M Y E V Y D A K S K S V S L					
1899	1908	1917	1926	1935	1944
CCA GTT CCA GAC TTG TGT GCA GTC TAT GCT GTT CAG GTG CGC TGT AAG AGG CTA					
P V P D L C A V Y A V Q V R C K R L					
1953	1962	1971	1980	1989	1998
GAT GGA CTG GGA TAT TGG AGT AAT TGG AGC AAT CCA GGC TAC ACA GTT GTC ATG					
D G L G Y W S N W S N P A Y T V V H					
2007	2016	2025	2034	2043	2052
GAT ATA AAA GTT CCT ATG AGA GGA CCT GAA TTT TGG AGA ATA ATT AAT GGA GAT					
D I K V P M R G P E F W R I I N G D					

Figure 1C

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2061	2070	2079	2088	2097	2106
ACT ATG AAA AAG	GAG AAA AAT	GTC ACT TTA	CTT TGG AAG	CCC CTG ATG	AAA AAT
T M K K	E K N V	T L L W	K P L M	K N	
2115	2124	2133	2142	2151	2160
GAC TCA TTG TGC	AGT GTT CAG	AGA TAT GTG	ATA AAC CAT	CAT ACT TCC	TGC AAT
D S L C	S V Q R	Y V I N	H H T S	C N	
2169	2178	2187	2196	2205	2214
GGA ACA TGG TCA	GAA GAT GTG	GGA AAT CAC	ACG AAA TTC	ACT TTC CTG	TGG ACA
G T W S	E D V G	N H T K	F T F L	W T	
2223	2232	2241	2250	2259	2268
GAG CAA GCA CAT	ACT GTT ACG	GTT CTG GCC	ATC AAT TCA	ATT GGT GCT	TCT GTT
E Q A H	T V T V	L A I N	S I G A	S V	
2277	2286	2295	2304	2313	2322
GCA AAT TTT AAT	TTA ACC TTT	TCA TGG CCT	ATG AGC AAA	GTA AAT ATC	GTG CAG
A N F N	L T F S	W P M S	K V N I	V Q	
2331	2340	2349	2358	2367	2376
TCA CTC AGT GCT	TAT CCT TTA	AAC AGC AGT	TGT GTG ATT	GTT TCC TGG	ATA CTA
S L S A	Y P L N	S S C V	I V S W	I L	
2385	2394	2403	2412	2421	2430
TCA CCC AGT GAT	TAC AAG CTA	ATG TAT TTT	ATT ATT GAG	TGG AAA AAT	CTT AAT
S P S D	Y K L M	Y F I I	E W K N	L N	
2439	2448	2457	2466	2475	2484
GAA GAT GGT GAA	ATA AAA TGG	CTT AGA ATC	TCT TCA TCT	GTT AAG AAG	TAT TAT
E D G E	I K W L	R I S S	S S V K	K Y Y	
2493	2502	2511	2520	2529	2538
ATC CAT GAT CAT	TTT ATC CCC	ATT GAG AAG	TAC CAG TTC	AGT CTT TAC	CCA ATA
I H D H	F I P I	E K Y Q	F S L Y	P I	
2547	2556	2565	2574	2583	2592
TTT ATG GAA GGA	GTG GGA AAA	CCA AAG ATA	ATT AAT AGT	TTC ACT CAA	GAT GAT
F M E G	V G K P	K I I N	S F T Q	D D	
2601	2610	2619	2628	2637	2646
ATT GAA AAA CAC	CAG AGT GAT	GCA GGT TTA	TAT GTA ATT	GTG CCA GTA	ATT ATT
I E K H	Q S D A	G L Y V	I V P V	I I	
2655	2664	2673	2682	2691	2700
TCC TCT TCC ATC	TTA TTG CTT	GGA ACA TTA	TTA ATA TCA	CAC CAA AGA	ATG AAA
S S S I	L L L G	T L L I	S H Q R	M K	
2709	2718	2727	2736	2745	2754
AAG CTA TTT TGG	GAA GAT GTT	CCG AAC CCC	AAG AAT TGT	TCC TGG GCA	CAA GGA
K L F W	E D V P	N P K N	C S W A	Q G	

Figure 1 D

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2763	2772	2781	2790	2799	2808
CTT AAT TTT CAG AAG AGA ACG GAC ATT CTT TGA AGT CTA ATC ATG ATC ACT ACA					
---	---	---	---	---	---
L N F Q K R T D I L * S L I M I T T					
2817	2826	2835	2844	2853	2862
GAT GAA CCG AAT GTG CCA ACT TCC CAA CAG TCT ATA GAG TAT TAG AAG ATT TTT					
---	---	---	---	---	---
D E P N V P T S Q Q S I E Y * K I F					
2871					
ACA TTC TGA AGA AGG 3'					
---	---	---	---	---	---
T F * R R					

Figure 1E

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/00880

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; C12N 15/12; C12Q 1/68; G01N 33/53

US CL : 435/6, 7.1, 7.2; 536/24.31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.2, 69.1, 252.3, 320.1; 436/501; 536/24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN/MEDLINE

search terms: leptin(2a)receptor#

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BARINAGA. Obesity: Leptin Receptor Weighs In. SCIENCE. 05 January 1996, Vol. 271, page 29.	1-12
Y	SCOTT. New chapter for the fat controller. NATURE. 11 January 1996. Vol. 379, pages 113-114, see entire document.	1-12
Y	TARTAGLIA et al. Identification and Expression Cloning of a Leptin Receptor, OB-R. Cell. 29 December 1995. Vol. 83, pages 1263-1271, see entire document.	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A document defining the general state of the art which is not considered to be of particular relevance	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E earlier document published on or after the international filing date	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* G	document member of the same patent family
* O document referring to an oral disclosure, use, exhibition or other means		
* P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

28 MARCH 1997

Date of mailing of the international search report

01 MAY 1997

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